STIC-ILL

ND

Fr n:
Sen :
T :
Subject:

Patten, Patricia
Tuesday, November 12, 2002 4:27 PM
STIC-ILL
reference for 09/486,239

420115

TI Inhibition of Listeria monocytogenes and Salmonella enteriditis by combinations of plant oils and derivatives of benzoic acid: the development of synergistic antimicrobial combinations.
AU Fyfe L; Armstrong F; Stewart J
CS Department of Dietetics and Nutrition, Queen Margaret College, Edinburgh, UK.
SO INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS, (1997 Jan) 9 (3) 195-9. Journal code: 9111860. ISSN: 0924-8579.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA Englsh

Thank you!

9552716

Patricia Patten
Patent Examiner
U.S. Patent and Trademark Office
-Biotechnology Center 1600-
Art Unit 1654
308-1189

COMPLETED

ELSEVIER

International Journal of Antimicrobial Agents 9 (1998) 195–199

INTERNATIONAL JOURNAL OF
Antimicrobial
Agents

Inhibition of *Listeria monocytogenes* and *Salmonella enteriditis* by combinations of plant oils and derivatives of benzoic acid: the development of synergistic antimicrobial combinations[1]

Lorna Fyfe [a,b,*], Fiona Armstrong [a,b], John Stewart [c]

[a] *Department of Dietetics and Nutrition, Queen Margaret College, Clerwood Terrace, Edinburgh EH12 8TS, UK*
[b] *Centre for Food Research, Queen Margaret College, Clerwood Terrace, Edinburgh EH12 8TS, UK*
[c] *Department of Medical Microbiology, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH8 9AG, UK*

Accepted 17 November 1997

Abstract

This study describes inhibitory properties of combinations of oil of fennel, oil of anise or oil of basil with either benzoic acid or methyl-paraben against *Listeria monocytogenes* and *Salmonella enteriditis*. Micro-organisms were cultured at 37°C in broth and viable counts measured over a 48-h period. *S. enteriditis* was particularly sensitive to inhibition by a combination of oil of anise, fennel or basil with methyl-paraben where there was < 10 CFU/ml after 1 h. *L. monocytogenes* was less sensitive to inhibition by each combination however there was a significant reduction in growth of 4–8 log by combinations of all oils and methyl-paraben at 8, 24 and 48 h. Synergistic inhibition by one or more combinations was evident against each micro-organism. © 1998 Elsevier Science B.V.

Keywords: Synergistic inhibition; Food-borne pathogens; Plant oils; Benzoic acid

1. Introduction

Listeria monocytogenes and *Salmonella enteriditis* are food-borne pathogens responsible for the significant increase in food-borne diseases observed over the past few years [1]. Susceptible individuals tend to be the very young, elderly and pregnant women who are particularly susceptible to listeriosis [2]. There are a variety of 'traditional' procedures used to control these micro-organisms, including good hygiene procedures and pressure and heat treatment [3], however there is little evidence of the widespread use of an effective synergistic combination preservative system to limit the spread of infection. Individual chemical preservatives such as organic acids [4] have been shown to inhibit food-borne pathogens and more recently, nisin, which is produced by *Lactococcus lactis*, has been shown to inhibit *L. monocytogenes* [5]. This microbe, unlike *S. enteriditis*, is capable of growth at 4°C and can readily achieve an infectious dose at this refrigeration temperature [6].

Inhibitory properties of plant oils have been known for some years [7] and recently Tassov et al. [8] described inhibition *of S. enteriditis* and *L. monocytogenes* in a model food system at 4 and 10°C by oil of mint.

Benzoic acid has been found as a 'natural' breakdown product in several milk-based products, such as yoghurt, milk and cheese, and is one of the first and most widely used preservatives in the world because it is relatively inexpensive and has low toxicity [9]. Furthermore, it has been shown to be inhibitory against food poisoning and spore forming bacteria [9] and has been shown to act synergistically with several components, such as sodium chloride and boric acid, against a variety of micro-organisms [10].

* Corresponding author. Tel.: + 44 131 173530; fax: + 44 131 3173528

[1] The work in this manuscript is the subject of a patent application.

PII S0924-8579(97)00051-4

Phenolic compounds such as methyl-paraben (esters of *p*-hydroxybenzoic acid) have been licensed for direct use in foods at a concentration not exceeding 0.1% [11]. 0.1% methyl-paraben has been shown to be weakly inhibitory towards *Staphylococcus aureus* and *Escherichia coli* but possesses synergistic antimicrobial properties when in combination with oil of fennel against these micro-organisms [12].

The work described in this paper indicates the potential of a combination of plant essential oil (oil of fennel, anise or basil) with either benzoic acid or methyl-paraben as effective inhibitors of food-borne pathogenic bacteria *S. enteriditis* and *L. monocytogenes*. Furthermore, several of these combinations are shown to exhibit synergistic antimicrobial properties which means that one or more components of the combination could be used at a relatively low concentration. This would clearly be useful in terms of cost, but more importantly could be more acceptable to the general public who have become increasingly critical of the use of chemical preservatives in food. In addition a combination containing a plant oil may be perceived by the general public as natural rather than chemical.

2. Materials and methods

2.1. Maintenance of bacteria

L. monocytogenes 11994 and *S. enteriditis* 4444 were purchased from the National Collection Type Culture, Porton Down, Wiltshire, UK. Each was stored for the long term in glycerol at −20°C, in the medium term on tryptone soya Agar (TSA) slopes and in the short term on TSA plates. All agar and broth (Tryptone Soya Broth) was purchased from Oxoid-Unipath, Basingstoke, UK).

2.2. Reagents

Oils of fennel, anise and basil were generously supplied by F.D. Copeland and Sons, Colanol House, 5 Westfield Street, London, UK. Benzoic acid, methyl-paraben and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich, Poole, UK.

2.3. Experimental procedure

An overnight TSB culture (10 μl) of *L. monocytogenes* or *S. enteriditis* incubated at 37°C was added to 10 ml of TSB containing 0.2% of oil of fennel, 0.2% oil of anise, 0.2% or 0.02% oil of basil in combination with 0.1% benzoic acid or 0.1% methyl-paraben. Corresponding controls were also prepared thus 10 μl of each overnight culture was added to the appropriate broth only. All tubes were incubated at 37°C. 1 ml samples were removed from each test and control after vortexing at 0, 1, 4, 8, 24 and 48 h and serially diluted in 0.01 M PBS. From each dilution, 100 μl was placed on an agar plate and spread across the surface. Plates were incubated at 37°C and colonies counted as colony forming units per ml (CFU/ml). As ethanol was used to prepare solutions of benzoic acid and methyl-paraben a diluent control was used where ethanol was added to each culture medium. The ethanol present was shown not to exhibit any inhibitory properties against micro-organisms tested. Due to the limitation of the serial dilution technique it was not possible to determine CFU of less than 10/ml.

Oils of fennel and anise were used at 0.2% and whilst oil of basil was originally used for comparative purposes at 0.2% it was later used at 0.02%. Benzoic acid and methyl-paraben were used at 0.1%. Several factors were considered when choosing these concentrations. Firstly, to determine the presence of synergistic inhibition by a combination it was necessary to use each component of the combination at a concentration which was relatively weakly inhibitory over the timescale of the experiment. Secondly, with respect to benzoic acid and methyl-paraben these cannot be used in foods in excess of 0.1% [11].

2.4. Reproducibility

Triplicate plates were prepared for each dilution and the mean count of the three plates was determined. The standard error of the mean was $\leq 10\%$. Each experiment was performed on three separate occasions. The mean count from the three separate experiments and the corresponding standard error of the mean which are shown in the results section.

2.5. Statistical test

Two-tailed paired Student's *t*-test was used to analyse inhibition caused by a combination compared to the corresponding control. Differences were judged to be statistically significant when $P < 0.05$.

2.6. Definition of synergy

The definition used is that of Eliopoulos and Moellering [13] thus inhibition is defined as synergistic when the combined preservatives demonstrate ≥ 1 $\log_{10}$ greater inhibition than the sum of the inhibitory effects of the preservatives used alone. This definition is also in agreement with Mims et al. [14].

Table 1
Inhibition of *L. monocytogenes* and *S. enteriditis* by combinations of plant oils and benzoic acid or methyl-paraben at 37°C over a period of 48 h.

Time	1 h	4 h	8 h	24 h	48 h
L. monocytogenes					
Control	6.46 ± 0.02	7.43 ± 0.04	8.63 ± 0.09	9.3 ± 0.03	9.06 ± 0.04
OA	6.13 ± 0.01	6.27 ± 0.04	6.64 ± 0.16	8.76 ± 0.01	8.96 ± 0.03
OF	5.9 ± 0.12	5.74 ± 0.11$^{\Delta}$	5.67 ± 0.29$^{\Delta}$	6.42 ± 0.41$^{\Delta}$	8.53 ± 0.33$^{\Delta}$
OB	3.42 ± 0.3$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$
BA	6.33 ± 0.11	7.22 ± 0.04	8.16 ± 0.06	9.09 ± 0.03	9.0 ± 0.06
MP	6.49 ± 0.01	7.02 ± 0.02	7.86 ± 0.05	9.27 ± 0.02	9.24 ± 0.02
OA/BA	6.23 ± 0.04	6.09 ± 0.1$^{\Delta}$	6.04 ± 0.16$^{\Delta}$	7.21 ± 0.36$^{\Delta}$	8.58 ± 0.13$^{\Delta}$
OA/MP	5.93 ± 0.05	5.71 ± 0.03$^{\Delta}$	5.27 ± 0.17$^{\Delta}$	4.16 ± 0.37$^{*,\Delta}$	2.5 ± 0.16$^{*,\Delta}$
OF/BA	5.96 ± 0.05	5.55 ± 0.10$^{\Delta}$	5.06 ± 0.42$^{\Delta}$	5.07 ± 0.27$^{*,\Delta}$	4.77 ± 0.36$^{*,\Delta}$
OF/MP	5.57 ± 0.22	5.3 ± 0.12$^{\Delta}$	4.18 ± 0.29$^{\Delta}$	3.64 ± 0.18$^{*,\Delta}$	1.99 ± 0.09$^{*,\Delta}$
OB/BA	2.77 ± 0.11$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$
OB/MP	3.1 ± 0.08$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$
OB'	6.15 ± 0.07	6.18 ± 0.16$^{\Delta}$	6.11 ± 0.24$^{\Delta}$	6.04 ± 0.18$^{\Delta}$	7.53 ± 0.22$^{\Delta}$
OB'/BA	6.10 ± 0.03	5.91 ± 0.09$^{\Delta}$	5.93 ± 0.09$^{\Delta}$	5.97 ± 0.57$^{\Delta}$	7.46 ± 0.20$^{\Delta}$
OB'/MP	6.09 ± 0.08	5.92 ± 0.06$^{\Delta}$	5.66 ± 0.88$^{\Delta}$	4.33 ± 0.18$^{*,\Delta}$	2.64 ± 0.18$^{*,\Delta}$
S. enteriditis					
Control	6.05 ± 0.02	6.81 ± 0.02	8.20 ± 0.06	9.04 ± 0.05	9.10 ± 0.04
OA	3.60 ± 0.21$^{\Delta}$	3.75 ± 0.28$^{\Delta}$	5.03 ± 0.36$^{\Delta}$	8.04 ± 0.1$^{\Delta}$	6.50 ± 0.50$^{\Delta}$
OF	4.22 ± 0.07$^{\Delta}$	4.20 ± 0.26$^{\Delta}$	5.65 ± 0.32$^{\Delta}$	8.36 ± 0.03$^{\Delta}$	5.13 ± 0.11$^{\Delta}$
OB	1.43 ± 0.05$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$
BA	6.02 ± 0.04	6.46 ± 0.01	7.32 ± 0.08	8.67 ± 0.14	8.52 ± 0.03
MP	6.04 ± 0.05	6.32 ± 0.03	6.63 ± 0.03	8.36 ± 0.05	8.45 ± 0.11
OA/BA	4.74 ± 0.16$^{\Delta}$	5.1 ± 0.32$^{\Delta}$	4.54 ± 0.26$^{\Delta}$	7.04 ± 0.02$^{\Delta}$	7.69 ± 0.08$^{\Delta}$
OA/MP	< 1.0$^{*,\Delta}$	< 1.0$^{*,\Delta}$	< 1.0$^{*,\Delta}$	< 1.0$^{*,\Delta}$	< 1.0$^{*,\Delta}$
OF/BA	4.49 ± 0.09$^{\Delta}$	4.42 ± 0.08$^{\Delta}$	5.53 ± 0.13$^{\Delta}$	7.93 ± 0.06$^{\Delta}$	7.25 ± 0.15$^{\Delta}$
OF/MP	< 1.0$^{*,\Delta}$	< 1.0$^{*,\Delta}$	< 1.0$^{*,\Delta}$	< 1.0$^{*,\Delta}$	< 1.0$^{\Delta}$
OB/BA	2.39 ± 0.18$^{\Delta}$	1.60 ± 0.18$^{\Delta}$	2.14 ± 0.11$^{\Delta}$	4.57 ± 0.33$^{\Delta}$	6.85 ± 0.09$^{\Delta}$
OB/MP	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$	< 1.0$^{\Delta}$
OB'	6.03 ± 0.05	6.83 ± 0.04	8.16 ± 0.08	8.97 ± 0.07	8.70 ± 0.03
OB'/BA	5.97 ± 0.03	6.42 ± 0.02	7.29 ± 0.04	8.79 ± 0.18	8.56 ± 0.02
OB'/MP	6.06 ± 0.03	6.24 ± 0.05	6.39 ± 0.12	7.90 ± 0.22	8.46 ± 0.03

The starting inoculum for *L. monocytogenes* was 6.45 ± 0.02 and for *S. enteriditis* it was 6.08 ± 0.03.
OA, oil of anise at 0.2%; OF, oil of fennel at 0.2%; OB, oil of basil at 0.2%; OB', oil of basil at 0.02%; BA, benzoic acid at 0.1%; MP, methyl-paraben at 0.1%.
Numbers represent the $\log_{10}$ of the mean of the count ± 1 SEM from three separate experiments. $\Delta = P < 0.05$ inhibition compared to that of corresponding control.
* Synergistic inhibition.

3. Results

3.1. Listeria monocytogenes

It can be seen from Table 1 that oil of basil at 0.2% was a potent inhibitor of this microbe where cells were undetectable (< 10 CFU/ml) at 4, 8, 24 and 48 h. Even at only 1 h of culture there was only 3.4 $\log_{10}$ CFU/ml which represents a reduction of 3.0 $\log_{10}$ compared to the corresponding control. Oil of fennel (0.2%) was less inhibitory although there was inhibition of growth by 1.7–3.0 $\log_{10}$ at 4, 8 and 24 h. Oil of anise, benzoic acid and methyl-paraben were not inhibitory over the 48 h.

For all combinations those containing methyl-paraben were more inhibitory than those containing benzoic acid and those containing oil of fennel were more inhibitory than those containing oil of anise. Thus, for oil of anise and methyl-paraben there was 6.6 $\log_{10}$ reduction in growth at 48 h compared to 0.5 $\log_{10}$ reduction for oil of anise and benzoic acid. For oil of fennel and benzoic acid there was 2–4.2 $\log_{10}$ inhibition of growth at times of 4 h and above, while the combination of oil of fennel and methyl-paraben exhibited extensive inhibition of growth after 8 h of 4.5 $\log_{10}$–7.1 $\log_{10}$ CFU/ml.

With respect to synergistic inhibition this was evident at 24 and 48 h for oil of anise with methyl-paraben and combinations of oil of fennel with benzoic acid or methyl-paraben. For example when oils were used with methyl-paraben there was over 6 $\log_{10}$ more inhibition than with additive inhibition with separate preservatives at 48 h. Even when oil of fennel was used with benzoic acid there was over 3 $\log_{10}$ more inhibition than with separate components at 48 h.

As mentioned previously, oil of basil (0.2%) was a potent inhibitor of *L. monocytogenes* in its own right and it was not possible to determine synergistic inhibition by combinations containing basil at this concentration. Oil of basil (0.02%) was however bacteriostatic over a 24-h period with a reduction in growth of 1.5 $\log_{10}$ at 48 h compared to the control. The combination of 0.02% oil of basil and benzoic acid exhibited the same pattern of inhibition as the oil on its own. Oil of basil and methyl-paraben inhibited growth at 24 and 48 h by more than 5.0 $\log_{10}$, indeed synergistic inhibition was detected at these times but at 48 h there was over 5.0 $\log_{10}$ more inhibition than with the separate preservatives.

3.2. *Salmonella enteriditis*

Individual oils at 0.2% and combinations of oils with benzoic acid or methyl-paraben were generally more inhibitory against *S. enteriditis* than *L. monocytogenes*. Oil of basil at 0.2% was the most potent oil with organisms being undetectable (< 10 CFU/ml) at all times except 1 h where there was only 1.4 $\log_{10}$ CFU/ml which represented a reduction of 4.6 $\log_{10}$ compared to the control. Oils of fennel and anise exhibited similar patterns of inhibition until 48 h when growth was reduced by 4.0 $\log_{10}$ and 2.6 $\log_{10}$, respectively. Benzoic acid and methyl-paraben exhibited little inhibition over the 48 h period.

As with *L. monocytogenes*, a combination containing methyl-paraben was more inhibitory than one containing benzoic acid. Thus for oils of fennel or anise in combination with methyl-paraben organisms were undetectable at all times. Furthermore synergistic inhibition was evident at all times but particularly after 24 and 48 h where there was over 6.0 and 3.0 $\log_{10}$ more inhibition than with the separate components. Synergistic inhibition was not evident with oils and benzoic acid where growth was reduced by 1–3 $\log_{10}$ at all times.

Microbes were undetectable in 0.2% oil of basil in combination with methyl-paraben at all times of culture. Oil of basil (0.2%) with benzoic acid was less inhibitory but there was significant reduction of growth at all times. Synergistic inhibition was not detected with any of these combinations. Oil of basil (0.02%) singly and in combination was weakly inhibitory at all times with synergy being undetectable.

4. Discussion

It has been recognised for some time that no single preservative will be completely effective against micro-organisms which contaminate food. Combination preservatives such as those described here, therefore seem preferable as they will have the potential to eliminate a broad range of microbes and will be more effective than single preservatives because of their multiple modes of action. Furthermore, as plant oils contain many active ingredients it is unlikely that micro-organisms would be able to mutate a sufficient number of genes to generate resistant strains for survival.

Synergistic effects by combinations of preservatives have been well documented however this is the first reported case of synergistic inhibition by combinations of plant oils and derivatives of benzoic acid against two of the major causes of food-borne illness namely *L. monocytogenes* and *S. enteriditis*. Oils used were fennel, anise and basil as they have similar active ingredients [15] whilst fennel has already been shown to synergistically inhibit a range of bacteria and yeast in combination with paraben [12]. Benzoic acid and methyl-paraben were used as their importance in combination preservative systems has been described previously [4,12].

In this study, combinations containing oil of fennel were, in the main, more inhibitory than those containing comparable concentrations of oil of anise or basil. Although all have estragole in common, oils of fennel and anise contain anethole but the increased activity of combinations containing fennel may be due to active ingredients which are unique to fennel such as fenchone and α-pinene [15].

Combinations containing methyl-paraben were more inhibitory than those containing benzoic acid. Phenolic compounds such as methyl-paraben are known to be more inhibitory than benzoic acid [16], however a drawback with this paraben is its increased toxicity and taste. These may not however be a problem within a combination which is synergistic (as most of the methyl-paraben containing combinations have been described in this study) as one or indeed both compounds of the combination could be used at a relatively low concentration.

It is clear from this study that *S. enteriditis* was more susceptible to inhibition by each combination than *L. monocytogenes*. It is unclear at this stage why this should be, but the obvious difference between these two micro-organisms is the structure of the cell wall and it may be that this is one or more of the targets of each combination. In this regard paraben and benzoic acid have been shown to disrupt bacterial cell walls [17] and it is reasonable to suggest that damage to the cell wall could then facilitate the uptake of oil so that it then reaches its intracellular target. This mechanism of synergy is in agreement with the permeabilization synergy theory of Denyer et al. [18]. The precise mode of action of each combination will be the subject of future investigations.

Much emphasis has been placed on the activity of each combination but it is important to point out the

potent activity of oil of basil on its own, where both micro-organisms were undetectable (< 10 CFU/ml) after only 4 h of culture in 0.2% of basil. It would be interesting to further determine the properties of this oil in both broth and food.

Although this study investigated the activity of combinations of plant oils and chemical preservatives over a 48-h period, at one pH (physiological pH) and only in laboratory medium, it does show the potential of these novel combinations as potent inhibitors *L. monocytogenes* but in particular *S. enteriditis*. Further studies will consider each of the above points, however emphasis will be placed on inhibitory experiments at lower temperatures such as 4 and 10°C. Indeed it has previously been mentioned that *L. monocytogenes* can grow at 4°C. More recently Jones et al. [19] have shown the fatty acid composition of the cell wall of this microbe to change at 4°C. It would be interesting to discover if these cells could be further inhibited by a combination at lower temperatures.

Acknowledgements

The authors wish to thank F.D. Copeland and Sons, Colanol House, 5 Westfield Street, London, UK, for their generous supply of plant oils. L. Fyfe wishes to thank the Research Committee of Queen Margaret College and the Department of Dietetics and Nutrition for their financial support of this project. The authors also thank Miss Susan Carr for typing this manuscript.

References

[1] Office for National Statistics. In: Monitor Population and Health. St. Catherine's House, 10 Kingsway, London WG2B 6JP, 1996;6.

[2] Farber JM, Peterkin PI. *Listeria monocytogenes* a food-borne pathogen. Microbiol Rev 1991;55(3):476–511.

[3] Gould GW. New Methods of Food Preservation. Glasgow: Chapman and Hall, 1995:1995.

[4] Doores S. Organic acids. In: Davidson PM, Branen AL, editors. Antimicrobials in Food. New York: Marcel Dekker, 1993:95–136.

[5] Delves-Broughton J. Nisin and its uses as a food preservative. Food Technol 1990;44:100–18.

[6] Walker SJ, Stringer MF. Growth of *Listeria monocytogenes* and *Aeromonas hydrophila* at chill temperatures. J Appl Bacteriol 1987;63:20.

[7] Deans SG, Ritchie G. Antibacterial properties of plant essential oils. Int J Food Microbiol 1987;5:156–80.

[8] Tassov CC, Drosinos EH, Nychas GJE. Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteriditis* and *Listeria monocytogenes* in model food systems at 4 and 10°C. J Appl Bacteriol 1995;78:593 600.

[9] Chipley JR. Sodium benzoate and benzoic acid. In: Davidson PM, Branen AL, editors. Antimicrobials in Food. New York: Marcel Dekker, 1993:11–48.

[10] Lueck E. Antimicrobial Food Additives. New York: Springer-Verlag, 1980.

[11] Code of Federal Regulations. In: Food and Drugs. Office of Federal Regulations. Washington DC: National Archives Records Services, General Services Administration, , 1991; parts 170–199.

[12] Hodgson I, Stewart J, Fyfe L. Inhibition of bacteria and yeast by oil of fennel and paraben: development of synergistic antimicrobial combinations. J Essential Oil Res 1998.

[13] Eliopoulos GM, Moellering RC. Antimicrobial combinations. In: Lorian V, editor. Antibiotics in Laboratory Medicine. Baltimore, MD: Williams and Wilkins, 1991:432.

[14] Mims CA, Playfair JHL, Roitt IM, Wakelin D, Williams R. Medical Microbiology. London: Mosby, 1993.

[15] Max B. This and that: the essential pharmacology of herbs and spices. TIPS 1992;13:15 20.

[16] Davidson PM. Parabens and phenolic compounds. In: Davidson PM, Branen AL, editors. Antimicrobials in Food. New York: Marcel Dekker, 1993:263–306.

[17] Freese E. Mechanism of growth inhibition by lipophilic acids. In: Kabara JJ, editor. Pharmacological Effects of Lipids. Champaign, IL: American Oil Chemists Society, 1978:123.

[18] Denyer SP, Hugo WB, Harding VD. Synergy in preservative combinations. Int J Pharm 1985;25:245–53.

[19] Jones CE, Shama G, Jones D, Roberts IS, Andrew PWC. Physiological and biochemical studies on psychotolerance in *Listeria monocytogenes*. J Appl Bacteriol 1997;83:31–5.